Hybrid Selection of cDNAs from 1 Megabase of Human Chromosome 19

Wufang Fan[1], *Greg Lennon*. Human Genome Center, L-452, Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA 94550. [1]Corresponding author.

The goal of this effort is to use cDNA hybrid selection techniques to isolate, sequence, and map coding regions located on human chromosome 19. Our initial experiments used flow-sorted chromosome 19 DNA and arrayed cDNA libraries, and have resulted in cDNA sublibraries enriched approximately 10 fold for sequences from chromosome 19. To increase the enrichment of the target genes, we are now selecting cDNAs hybridizing to cosmid contigs from the physical map of chromosome 19 (see other posters for physical map details).

Two contigs spanning in total 1 megabase have been studied. A contig of 400 kb located within 19p12-13.1, and containing the gene defective in pseudoachondroplasia and multiple epiphyseal dysplasia, was the first on which our protocols were optimized. Pooling the results from selection experiments with three different cDNA libraries, 55% of the selected cDNA fragments map back specifically to the starting contig. Even though the cosmids lack ribosomal sequences, a major contaminant of the remaining 45% of selected clones are sequences homologous to rRNA. After sorting the cDNAs based on both hybridization and sequencing, 17 distinct genes have been characterized. We have also compared these 17 genes with the results of an exon-trapping experiment conducted in parallel using the same cosmids. A second contig spanning 600 kb of chromosome 19q13.1 has recently been used as the basis for hybrid selection experiments. By choosing cDNA material with low amounts of rRNA contamination, we have been able to increase the percentage of selected fragments mapping back to the starting cosmids to 65-75%, even after only one round of selection. These results indicate that the rate-limiting steps in isolating genes from a region are now increasingly the ability to identify different cDNA fragments derived from the same gene, and the isolation of corresponding full-length cDNAs.

(This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under contract no. W-7405-ENG-48.)